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(54) [Title of the Invention]

Method for powderization of water-soluble phospholipid

(57) [Abstract]

[Constitution] A water-soluble phospholipid is subjected to an azeotropic dehydration with an alcohol having 1 to 4 carbon atoms and a non-polar solvent to a water content of 3 wt% or less and, after that, is subjected to crystallization utilizing an alcohol having 1 to 4 carbon atoms and a non-polar solvent in a specified weight ratio.

[Advantage] A powder of water-soluble phospholipid can be obtained with a high yield by a simple operation.

[Claims]

[Claim 1]

A powderization method for a water-soluble phospholipid comprising the following steps (1) and (2):

(1) dissolving a water-soluble phospholipid in an alcohol having 1 to 4 carbon atoms, then adding a non-polar solvent mutually soluble with the alcohol, and executing an azeotropic dehydration to reduce the water content of the water-soluble phospholipid to 3 wt% or less; and

(2) dissolving the water-soluble phospholipid obtained in the step (1) in an alcohol having 1 to 4 carbon atoms in an amount of 1 to 5 times by weight, then adding a non-polar solvent in an amount of 10 to 30 times by weight of the water-soluble phospholipid, and causing crystallization at a temperature of 0 to 30°C, so as to obtain a water-soluble phospholipid in powder form.

[Detailed Description of the Invention]

[0001]

[Industrial Application Field]

The present invention relates to a method of powderization of a water-soluble phospholipid.

[0002]

[Conventional Art]

A glycerophospholipid of a high purity, being suitable for application to cosmetics, pharmaceuticals and the like, can be obtained by acylating 1-position and 2-position of a water-soluble phospholipid with an acylating agent such as a fatty acid anhydride or a fatty acid chloride. However, since such

acylation is normally executed in a non-polar solvent, it is necessary, in order to improve an acylation rate, to use the water-soluble phospholipid, which is insoluble in the non-polar solvent, in powder form.

[0003]

For powderization of water-soluble phospholipid, the following methods have been proposed:

(a) a method of forming a glycerophosphatidyl choline-cadmium chloride complex (H. Brockerhoff, M. Yorkowski, Canadian Journal of Biochemistry, Vol. 43, p1777, 1965);

(b) a method of carrying glycerophosphatidyl choline on a metal oxide, an alkali metal salt or an alkali earth metal salt of a higher fatty acid, an inorganic neutral salt or the like (Japanese Patent Laid-Open No. 1-131190); and

(c) a method of carrying glycerophosphatidyl choline on silica gel (Japanese Patent Laid-Open No. 2-101086).

[0004]

Any of these methods achieves the powderization utilizing a carrier, but involves problems such that the carrier is toxic or the carrier is difficult to be removed after the acylation.

[0005]

[Problems to be Solved by the Invention]

An object of the present invention is to provide a powderization method, capable of solving the problems in the aforementioned conventional technologies and of producing a powder of a water-soluble phospholipids, being suitable as a raw material of cosmetics, pharmaceuticals and the like, by a simple operation.

[0006]

[Means for Solving the Problems]

The present invention is a powderization method for a water-soluble phospholipid including the following steps (1) and (2):

(1) dissolving a water-soluble phospholipid in an alcohol having 1 to 4 carbon atoms, then adding a non-polar solvent mutually soluble with the alcohol, and executing an azeotropic dehydration to reduce the water content of the water-soluble phospholipid to 3 wt% or less; and

(2) dissolving the water-soluble phospholipid obtained in the step (1) in an alcohol having 1 to 4 carbon atoms in an amount of 1 to 5 times by weight, then adding a non-polar solvent in an amount of 10 to 30 times by weight of the water-soluble phospholipid, and causing crystallization at a temperature of 0 to 30°C, so as to obtain a water-soluble phospholipid in powder form.

[0007]

The water-soluble phospholipid to be employed as the raw material in the present invention may be synthesized by an ordinary method such as a chemical synthesis starting from D-mannitol, a method of hydrolyzing a natural phospholipid with a chemical, or a method of hydrolyzing a natural phospholipid with phospholipase, but a water-soluble phospholipid having a purity of 90% or higher is preferable. Examples of the water-soluble phospholipid include glycerophosphatidyl choline, glycerophosphatidyl ethanolamine, glycerophosphatidyl serine, glycerophosphatidyl inositol, and glycerophosphatidyl glycerol.

[0008]

The step (1) in the present invention is a step of dissolving a water-soluble phospholipid in an alcohol having 1 to 4 carbon atoms, then adding a non-polar solvent mutually soluble with the alcohol, and executing an azeotropic dehydration to reduce the water content of the water-soluble phospholipid to 3 wt% or less. Examples of the alcohol having 1 to 4 carbon atoms include methanol, ethanol, propanol, isopropanol, and butanol. The non-polar solvent is only required to be mutually soluble with the alcohol having 1 to 4 carbon atoms, and is preferably chloroform, dichloromethane or carbon tetrachloride.

[0009]

An amount of the alcohol having 1 to 4 carbon atoms is preferably from 1 to 5 times of the weight of the water-soluble phospholipid, and an amount of the non-polar solvent is preferably from 5 to 10 times of the weight of the alcohol having 1 to 4 carbon atoms. Execution is possible even outside these ranges, but in such case, a long time is required for the azeotropic dehydration or the production efficiency is lowered.

[0010]

The azeotropic dehydration may be executed in a repeated manner, for example, with an evaporator or the like, but is preferably executed in a four-necked flask and the like at 20 to 60°C, under a reduced pressure, and under distilling-off of the solvent. In such case, it is efficient to execute the process in a continuous manner, by adding the alcohol having 1 to 4 carbon atoms and the non-polar solvent, in amounts matching the amounts of distilled-off solvents.

[0011]

The water content of the water-soluble phospholipid is 3 wt% or less. The powderization becomes difficult when the water content exceeds 3 wt%.

[0012]

The step (2) of the present invention is a step of dissolving the water-soluble phospholipid obtained in the step (1) in an alcohol having 1 to 4 carbon atoms in an amount of 1 to 5 times by weight, then adding a non-polar solvent in an amount of 10 to 30 times by weight of the water-soluble phospholipid, and causing crystallization at a temperature of 0 to 30°C, so as to obtain a water-soluble phospholipid in powder form.

[0013]

The alcohol having 1 to 4 carbon atoms and the non-polar solvent may be same as those employed in the step (1). An amount of the alcohol having 1 to 4 carbon atoms is from 1 to 5 times of the weight of the water-soluble phospholipid, and, with an amount less than this range, the water-soluble phospholipid becomes difficult to dissolve, while the yield becomes lower with an amount exceeding this range. An amount of the non-polar solvent is from 10 to 30 times of the weight of the water-soluble phospholipid, and the yield is lowered with an amount less than this range, while the production efficiency is lowered with an amount exceeding this range.

[0014]

The crystallized water-soluble phospholipid is obtained as a powder by filtration followed by distilling off the solvent in a

vacuum dryer, and this operation is preferably executed in an atmosphere of an inert gas such as nitrogen gas.

[0015]

[Effects of the Invention]

The present invention allows to obtain the water-soluble phospholipid in powder form in a simple manner and with a high yield. The obtained water-soluble phospholipid in powder form, not involving a carrier, is suitable as a raw material for cosmetics, pharmaceuticals and the like. Also the powderization method for the water-soluble phospholipid of the present invention also provides a purifying effect to remove non-water-soluble substances such as unreacted phospholipids, lysophospholipids, and fatty acids.

[0016]

[Examples]

In the following, the present invention will be further described specifically by Examples and Comparative Examples. The purity of phospholipid was measured by a thin layer chromatography (hereinafter represented as TLC). The TLC was conducted by utilizing Kieselgel 60 (Merck & Co., Ltd.), spotting 2 μL of a 5 wt% sample, executing a development with chloroform : methanol : distilled water : 28% ammonia water = 65 : 25 : 4 : 0.3 (weight ratio), and spraying 10 g of copper sulfate (anhydrous) and 8 ml of phosphoric acid (85%) diluted to 100 ml with distilled water, followed by heating.

[0017]

Example 1

Egg yolk lecithin was hydrolyzed with tributylammonium hydroxide to obtain glycerophosphatidyl choline of a purity of 95 wt%. It had a water content of 8.2 wt%. The obtained glycerophosphatidyl choline (10 g) were charged in a 300-ml four-necked flask, and dissolved in 30 g of methanol, then 150 g of chloroform were added, and an azeotropic dehydration was conducted by distilling off the solvents at 40°C under a reduced pressure with stirring, and adding solvents (methanol/chloroform = 1/5 (weight ratio)) in an amount matching the distilled-off amount. After complete distilling-off of the solvents, the water content measured by Karl-Fischer method was 1.2 wt%.

[0018]

The glycerophosphatidyl choline was dissolved in 30 g of methanol, then 150 g of chloroform were added, and the mixture was cooled to 5°C and maintained at this temperature for 2 hours. The precipitated powder was separated by filtration in a nitrogen gas atmosphere, and the solvent was distilled off at 40°C and under a reduced pressure to obtain 9.1 g of glycerophosphatidyl choline in powder form. The yield was 91 wt% and the purity was 99 wt%.

[0019]

Example 2

Hydrogenated soybean lecithin was hydrolyzed with phospholipase A1 and phospholipase A2 to obtain glycerophosphatidyl choline of a purity of 92 wt%. The water content was 6.6 wt%. The obtained glycerophosphatidyl choline (10 g) were dissolved in 20 g of ethanol, then 200 g of dichloromethane were added, and distilling-off of solvent at 40°C

in an evaporator was repeated three times. The water content was 0.8 wt%.

[0020]

The glycerophosphatidyl choline was dissolved in 20 g of ethanol, then 200 g of dichloromethane were added, and the mixture was processed thereafter in the same manner as in Example 1 to obtain 8.8 g of glycerophosphatidyl choline in powder form. The yield was 88 wt% and the purity was 97 wt%.

[0021]

Example 3

Phosphatidyl serine extracted from bovine brain was hydrolyzed with tributylammonium hydroxide to obtain glycerophosphatidyl serine with a purity of 94 wt%. The water content was 11.3 wt%. The obtained glycerophosphatidyl serine (1 g), propanol (3 g) and carbon tetrachloride (20 g) were subjected, in a 50-ml four-necked flask, to an azeotropic dehydration in the same manner as in Example 1. The water content was 2.1 wt%.

[0022]

The glycerophosphatidyl serine was processed in the same manner as in Example 1, except for employing 3 g of propanol and 20 g of carbon tetrachloride to obtain 9.0 g of glycerophosphatidyl serine in powder form. The yield was 90 wt% and the purity was 98 wt%.

[0023]

Example 4

Phosphatidyl ethanolamine extracted from egg yolk phospholipid was hydrolyzed with tributylammonium hydroxide to

obtain glycerophosphatidyl ethanolamine of a purity of 91 wt%. The water content was 7.8 wt%. The obtained glycerophosphatidyl ethanolamine (1 g) was dissolved in 3 g of methanol, then 15 g of chloroform were added, and an azeotropic dehydration was conducted in the same manner as in Example 3. The water content was 1.2 wt%.

[0024]

Then 3 g of methanol and 15 g of chloroform were used, in the same manner as in Example 3, to obtain 8.9 g of glycerophosphatidyl ethanolamine in powder form. The yield was 89 wt% and the purity was 99 wt%.

[0025]

Comparative Example 1

Glycerophosphatidyl choline (10 g) the same as in Example 1 (water content 8.2 wt%) were dissolved in 30 g of methanol, then 200 g of chloroform were added, and the mixture was cooled to 5°C and maintained at the same temperature for 4 hours, but powder of glycerophosphatidyl choline could not be obtained. Powderization was not possible, because the water content exceeded 3 wt%.

[0026]

Comparative Example 2

Glycerophosphatidyl choline (10 g) the same as in Example 1 (water content 8.2 wt%) were dissolved in 100 g of water, and lyophilization was conducted for 10 hours, which provided glycerophosphatidyl choline in paste but could not provide glycerophosphatidyl choline in powder form. The powderization was not possible by lyophilization.

[0027]

Comparative Example 3

Glycerophosphatidyl choline, subjected to azeotropic dehydration in the same manner as in Example 1 were dissolved in 100 g of methanol, then 200 g of chloroform were added and the mixture was cooled to 5°C, but there was no precipitate even after 4 hours. Powder could not be obtained as the amount of alcohol having 1 to 4 carbon atoms exceeded the range of the present invention.